Chapter

Technological Advances in Synthetic Biology for Cellulosic Ethanol Production

Antonio Luiz Fantinel, Rogério Margis, Edson Talamini and Homero Dewes

Abstract

The resurgence of biofuels in the recent past has brought new perspectives for renewable energy sources. Gradually the optimistic scenarios were being challenged by the competition for raw materials dedicated to direct or indirect human food. Second-generation biorefineries have emerged as technological alternatives to produce biofuels from lignocellulosic biomass. The third generation of biorefineries uses alternative raw materials like algae and microalgae. Despite the technical feasibility, these biorefineries were indebted for their economic performance. Synthetic biology has provided new microbial platforms that are increasingly better adapted to industrial characteristics to produce biofuels and fine chemicals. Synthetic biology bioengineers microorganisms to take advantage of the low-cost and less-noble raw materials like lignocellulosic biomass, carbon dioxide, and waste as a sustainable alternative for bioenergy generation using bio-substrates. In this chapter, we analyze the innovations in synthetic biology as applied to cellulosic ethanol production based on registered patents issued over the last twenty years (1999–2019). Using Questel-Orbit Intelligence, we recovered a total of 298 patent families, from which we extracted the key concepts and technology clusters, the primary technological domains and applications, the geographical distribution of patents, and the leading patents assignees. Besides, we discuss the perspectives for future research and innovations and the market and policy opportunities for innovation in this technological field. We conclude that the patented technologies serve as a proxy for the development of synthetic biotechnology applied in cellulosic ethanol production by the fourth generation of biorefineries.

Keywords: Metabolic engineering, microorganisms, CRISPR, advanced biofuels, genetic engineering

1. Introduction

The transition from a fossil resource-based economy to a bio-based economy necessarily goes using synthetic biotechnologies [1–3]. Synthetic biology has been evolving and positively affecting human life by providing the opportunity to design and build new biological parts, devices, and systems that do not exist or redesign existing biological systems [4, 5] to produce biofuels and other chemicals [6, 7].

The overlap between synthetic biology and bioeconomy occurs when we consider the latter part of the economy that uses new biological knowledge for commercial and industrial purposes, improving human well-being [8]. This perception intensifies when we ponder the sustainable use of biomass for non-food-biofuel production [9, 10].

Currently, ethanol produced from sugarcane in Brazil [11] and corn in the US [12] is the main alternative in the global supply chain of renewable fuels as a substitute for gasoline. However, this phenomenon fosters scientific debates about land use and food security, given that these are raw materials based on starch and sugar and that can be intended, directly or indirectly, for human food [13, 14].

In microbial ethanol production from lignocellulosic biomass, the dependence on food-related feedstocks is overcoming, being a sustainable alternative for bioenergy generation using substrates from the bioeconomy world [15, 16]. Lignocellulosic biomass is one of the most abundant feedstocks on the globe [17], with a production of approximately 181.5 billion tons/year [18], and can bring about significant changes in socioeconomic, agricultural, and energy systems when efficiently employed [19].

To overcome critical steps of microbial fermentation processes and to increase yields, technological advances are necessary on synthetic biology tools like metagenomics [20], genetic engineering [21], orthogonal communication systems [22], metaproteomics [23], metabolomics [24], and metabolic engineering [25–27].

From an industrial point of view, investment in these technological solutions depends upon the technical feasibility of using a particular organism to produce a specific compound and on the economic feasibility that results in profitable activity in the long run. Low yields in industrial processes are still recurrent due to low cell density, slowing down the efficient industrial expansion of this field [28–32] and are critical for biofuel production. Competitive synthetic biology technologies for ethanol production are estimated to be available in the coming years [33].

In the present chapter, we analyze the applications of synthetic biology tools related to cellulosic ethanol production from registered patents, visualizing the technological trends and their regional, institutional, and R&D markets distribution in the years 1999–2019. Patent analysis is one of the approaches to access innovative technologies and commercial aspects of a specific field [34]. The interest in searching for patents on cellulosic ethanol [35–38] or synthetic biology [39–41] is expanded here towards the technological development to future energy needs guided by the sustainable bioeconomy agenda. A total of 298 patent families were retrieved using Questel-Orbit Intelligence software. From them, we provide a high-quality dataset from the Questel-Orbit database that can contribute to formulating strategies and policies geared towards the development of these technologies and their applications in emerging markets, ensuring bioeconomic development for the next generations [33].

2. The evolution of innovations in synthetic biology and cellulosic ethanol

Figure 1 shows the distribution of the 298 synthetic biology patent families related to cellulosic ethanol throughout the twenty-year interval 1999–2019. The values correspond to the total frequencies following the International Patent Classification (IPC). In the eight-year interval 1999–2006, the annual number of published patents was negligible, with the first patent application occurring only in 2001. From 2007 to 2011, the number of applications on this topic showed

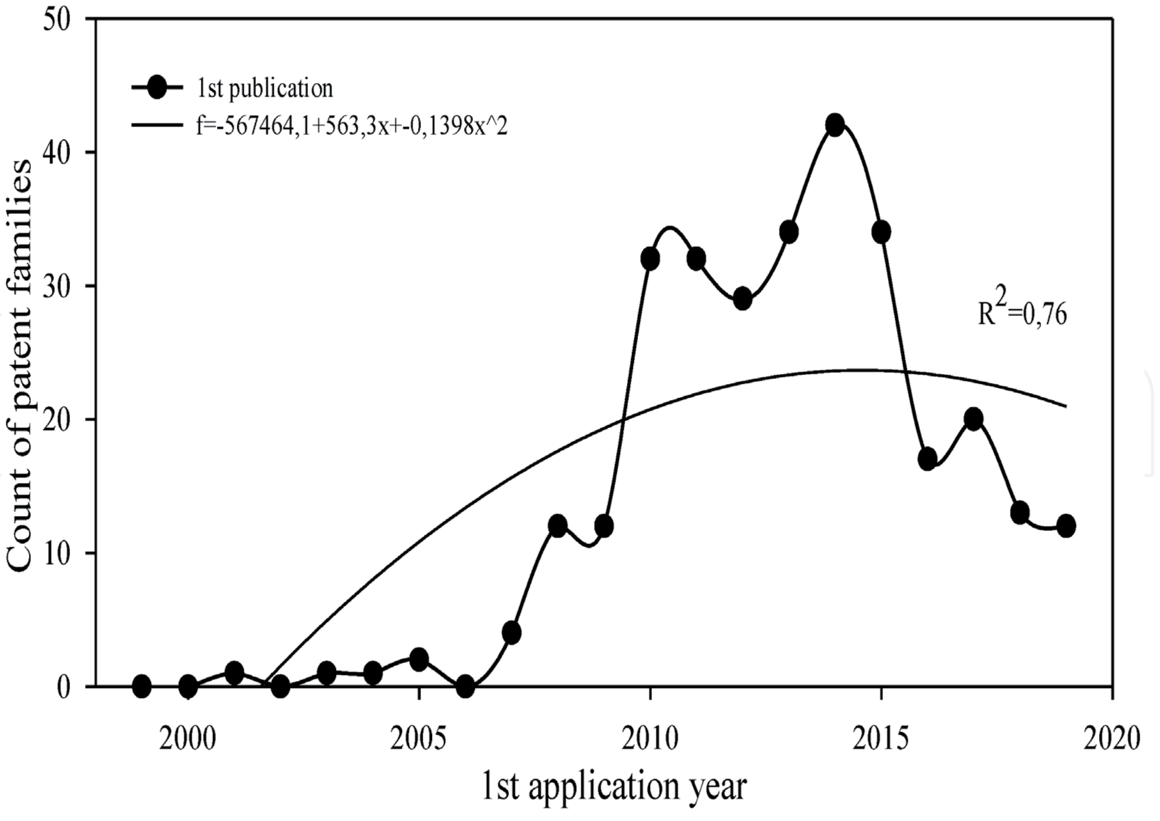

Figure 1.
Frequency of synthetic biology patents related to cellulosic ethanol. Source: Research data from Questel-orbit platform.

a considerable increase, peaking in 2010 and 2011. Subsequently, applications decreased in 2012, before temporarily recovering in 2013 and 2014. After that year, the applications decreased considerably, as observed through the trend line of synthetic biology patent applications for cellulosic ethanol. Thus, from 2015 there is a decline and subsequent stabilization, which we can understand as a transition from growth to maturity [42] or a consequence of time lag between patent application and patent grant.

2.1 Key concepts and technology clusters

The distribution of the main concepts among the retrieved patent families is presented in **Figure 2**. Nine semantic clusters regularly used by patent applicants were identified (**Figure 2A**). Most of these patents are related to the use of microorganisms (yeast and gram-negative bacteria), enzymatic activity, biomass, fermentation product, and biofuel production. As for the application of the technologies, we found the predominance of raw materials from biomass, such as agricultural residues (corn straw, wheat, rice, sugarcane bagasse, and switchgrass) and their main fermentable sugars (xylose, hemicellulose, and arabinose) for ethanol production. In the first years, the term "xylose" appeared more prominently than the others (**Figure 2B**), followed by "corn stover" and "lignocellulosic biomass". In the sequence, these terms were accompanied by the words "ethyl alcohol" given that the field includes this focus.

Therefore, by identifying the concepts commonly employed in the field of synthetic biology concerning cellulosic ethanol, we can propose insights for the development or identification of protected technologies in an emerging technological field with a view to its industrial application.

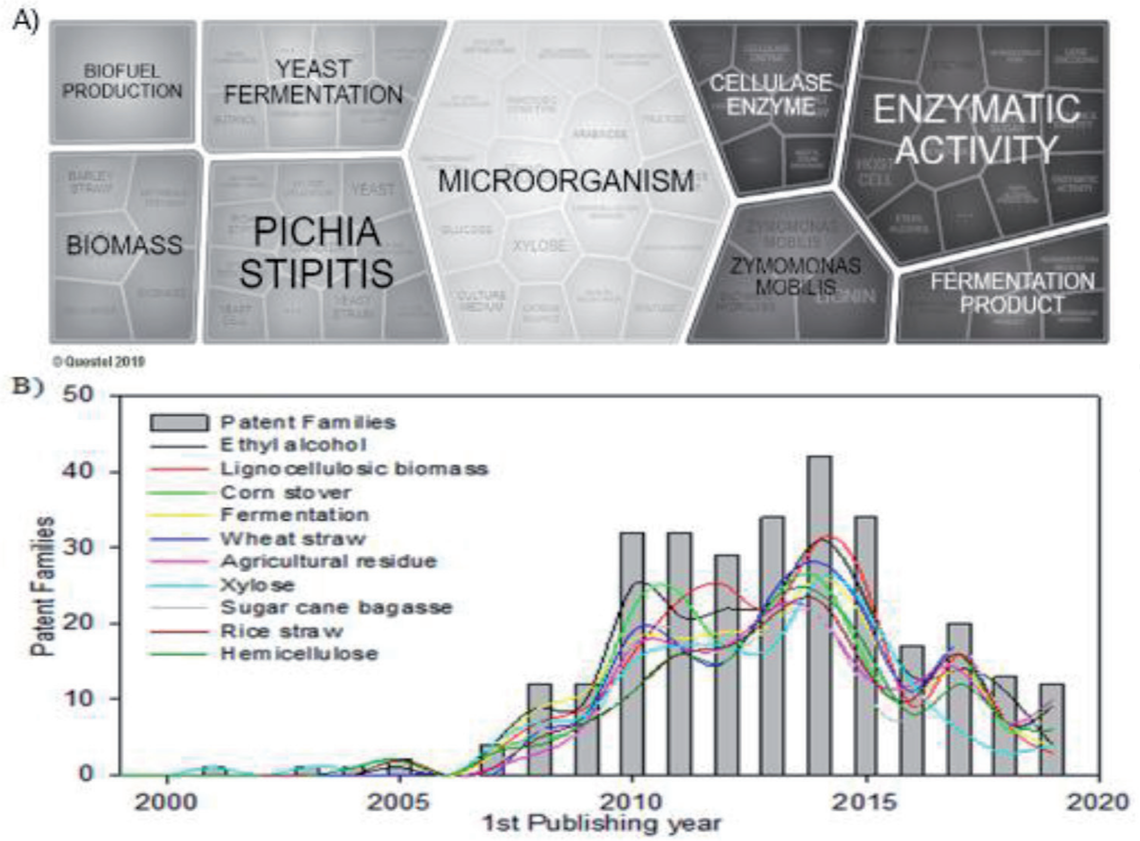

Figure 2.
Prevailing concepts in the retrieved patent families. 2A provides an overview of the content of this portfolio formed from the 298 synthetic biology patent families for cellulosic ethanol. 2B shows the distribution of these concepts concerning the complete portfolio over the twenty years surveyed. Source: Research data from Questel-orbit platform.

2.2 Major technological domains and applications

To elucidate the main technological domains and applications of the patent families, we analyzed the codes predominantly used to classify them (**Table 1**) following the structure proposed by IPC [43]. Approximately 40% of synthetic biology patent families related to cellulosic ethanol belong to the C12P classes. This class contemplates inventions concerning fermentation processes or using enzymes to synthesize a desired chemical composition or compound or to separate optical isomers of a racemic mixture. Within this class, group C12P-007, related to the preparation of organic compounds, represents 37% of the patents. Next in importance is class C12N (30% of the patent families), which deals with microorganisms or enzymes, or compositions thereof; propagation, preservation, or maintenance of microorganisms; genetic or mutation engineering; and culture media. Group C12N-001 accounts for 20% of the patents in this class, concerning processes for propagation, maintenance, or preservation of microorganisms or their compositions, or preparation, isolation of compositions containing a microorganism, and culture media for such.

When analyzed separately for each of the predominant codes (**Table 1**), we see the prominence of code C12P-007/06 (29 patent families). This code is related to the preparation of organic compounds containing oxygen, such as fuel ethanol, whose preponderant claims include yeast capable of fermenting xylose in the presence of glucose [44, 45], development of pretreated biomass [46, 47], use of yeast and bacteria in the presence of glycerol [48]. Among other technologies, we identified inventions related to methods for engineering *Thermoanaerobacterium saccharolyticum* [48], bioprocessing using recombinant *Clostridium* [48], methods for ethanol

IPC Codes	Code description	Family frequency
C12P-007/06	Preparation of oxygen-containing organic compounds [2006.01] • containing a hydroxy group [2006.01] •• acyclic [2006.01] ••• Ethanol, i.e. non-beverage [2006.01]	29
C12P-007/10	•••• produced as by-product or from waste or cellulosic material substrate [2006.01] ••••• substrate containing cellulosic material [2006.01]	20
C12N-001/21	• Bacteria; Culture media therefor [2006.01] •• modified by introduction of foreign genetic material [2006.01]	13
C12P-007/16	••• Butanols [2006.01]	13
C12N-001/20	• Bacteria; Culture media therefor [2006.01]	10
C12N-015/81	• Recombinant DNA-technology [2006.01] •• Introduction of foreign genetic material using vectors; Vectors; Use of hosts therefor; Regulation of expression [2006.01] ••• Vectors or expression systems specially adapted for eukaryotic hosts [2006.01] •••• for fungi [2006.01] ••••• for yeasts [2006.01]	10
C12P-007/18	••• polyhydric [2006.01]	9
C12P-007/14	•••• Multiple stages of fermentation; Multiple types of microorganisms or reuse for microorganisms [2006.01]	9
C12N-001/19	• Fungi (culture of mushrooms A01G 18/00; as new plants A01H 15/00); Culture media therefor [2006.01] •• Yeasts; Culture media therefor [2006.01] ••• modified by introduction of foreign genetic material [2006.01]	8
C12N-001/22	• Processes using, or culture media containing, cellulose or hydrolysates thereof [2006.01]	7

Source: research data from Questel-Orbit platform.

Table 1.
Leading IPC codes for synthetic biology patent families for cellulosic ethanol.

and hydrogen production using microorganisms [49], methods for propagation of microorganisms for hydrolysate fermentation [50], development of fermentation processes using transketolase/thiaminapyrophosphate enzymes [51], and hydrolysis of cellulosic material augmented with an enzyme composition [52].

Accordingly, code C12P-007/10 specifically addresses waste or cellulosic material or substrate containing cellulosic material for ethanol production and accounts for twenty (20) major patent families. These inventions relate to the development of microorganisms fermenting xylose and arabinose into ethanol [53], co-fermentation of pretreated lignocellulosic biomass [54, 55], wet oxidation methods of biomass [56], use of genetic engineering in microorganisms and enzymes [57].

The development of bacteria and culture mediums modified by the introduction of exogenous genetic material is classified by codes C12N-001/20 and C12N-001/21, which together represent twenty-three (23) major patent families. The inventions relate to the development and adaptation of *Zymomonas mobilis* strains [58], *C. thermocellum* [59, 60], *E. coli* [60], and anaerobic thermophilic bacteria [61, 62] for ethanol production. In addition, we verified the existence of technologies for the removal or inactivation of microbial inhibitors in biomass hydrolysates [63] and the conversion of xylose [64, 65] and arabinose [65] into ethanol.

Preparation of oxygen-containing organic compounds to produce butanol (C12P-007/16) features ten (10) patent families. The technologies pertinent to this code are related to recombinant microbial host cells of *S. cerevisiae* capable of converting hemicellulosic material into butanol-like alcohols [66], separation of undissolved solids after liquefaction [66] co-production of biofuels [66], microorganisms using protein and carbohydrate hydrolysates from biomass [67], and genetic engineering in bacteria [67] and yeast [68].

The use of recombinant DNA technologies via vectors and expression regulation in yeast and fungi categorized by code C12N-015/81 presents ten (10) main patent families. These patents target the development of yeast cells with xylose isomerase activity [69], culture medium and bioreactors [70], L-arabinose transporter polypeptide (I) from *Pichia stipitis* [70], gene transcription control [70], glycerol-free ethanol production using recombinant yeast [71, 72], microbial cells capable of transporting xylo-oligosaccharides [72], and yeast cells with a reduced enzyme activity for NADH-dependent glycerol synthesis [72].

The preparation of organic compounds containing at least two hydroxyl groups (C12P-007/18) features nine (9) main patent families. In this code, inventions are directed to the development of non-native pentose metabolic pathways in yeast cells [73], yeast genes encoding enzymes in the pentose pathway [74], genetically modified thermophilic or mesophilic microorganism [74], *S. cerevisiae* strains with reduced glycerol productivity [74] and fermentation microorganism propagation [75].

New forms of fermentation through multiple stages, different types of microorganisms, or reuse of microorganisms represented by code C12P-007/14 present nine (9) main patent families. The technologies are related to the production of syrups enriched with C5 and C6 sugars [75], ethanol production from lignocellulosic biomass [76] and xylitol production from biomass with enriched pentose component [77]. Methods for pectin degradation [78], pretreated cellulosic material [78], biocatalyst development [79] and microorganism propagation [80] for ethanol production are also checked in this code.

Yeast modification by introducing exogenous genetic material represented by C12N-001/19 features eight (8) main patent families. The inventions relate to the use of metabolic engineering for the elimination of the glycerol pathway [78], joint utilization of xylose and glucose [78], and rapid fermentation of xylose [78] in yeast. Methods for enhanced expression of a glycolytic system enzyme [78], glycerol transport [81] and alpha-ketoisovalerate conversion to isobutyraldehyde [81] also integrate this code.

The tenth code with seven (7) patent families relates to processes using culture medium containing cellulose or hydrolysates (C12N-001/22). The inventions concern continuous xylose growth using *Zymomonas* [81], oligosaccharide degradation by recombinant host cells [82] and lignocellulose bioprocessing employing recombinant *Clostridium* [83]. Methods for glycerol reduction in biomass fermentative processes [83], increasing tolerance to acetate toxicity in recombinant microbial host cells [84] and controlling contamination during fermentation [84] also integrate this code.

The knowledge present in these technological domains and their applications allows researchers to identify potential fields of development of new cellulosic ethanol production routes using synthetic biology as a technical platform.

2.3 The geographical distribution of innovations

Next, we analyzed the geographical distribution of synthetic biology patent families related to cellulosic ethanol, according to priority country (**Figure 3**). We found only 14 priority countries holding this technology. The lead-in technological innovation in this field is the USA (**Figure 3**) since approximately 67% of the patents recovered are in the name of American applicants. The main technological applications patented by American inventors are focused on the production of ethanol from biomass by-products or wastes (C12P-007/10; C12P-007/06), as well as modification of bacteria (C12N-001/21) and fungi (C12N-001/19) by introducing endogenous genetic material as applications to overcome the current barriers to the conversion of biomass to ethanol [85, 86]. Following at a distance is the European Patent Organization, followed by Japan and China, which account for 13%, 6%, and 3% of patent families, respectively. The remaining patent families, which total 11%, are distributed among ten other countries.

The global distribution of patent families protected in the various offices can be seen in **Figure 4**. The data corroborates the identification of target markets and demonstrates the patenting strategies of the applicant countries. The illustration confirms that demand is concentrated in the United States, with 49% of patent families, followed by the European Patent Organization (37% of families), India (33% of families), Brazil (30% of families), and China (29% of patent families).

Through this data, the strategies for patent protection used by applicants in the sector studied are identified. The preference for registration in patent offices in certain countries indicates the potential of the markets from the viewpoint of the need for commercial protection of new industrial technologies.

2.4 Leading patent assignees

The main assignees of patents in synthetic biology associated with cellulosic ethanol production encompass both private companies and educational and research institutions. The applicants were analyzed by the number of active patents, the average size of these families, generality index, and originality (**Table 2**). The number of patents and the average size of patent families refer to active patents and their breadth, respectively. In turn, the generality index is defined by Hall et al. [87] as the range of fields of future citations of a given patent. Future citations can be used to assess the subsequent generations of an invention that have benefited from an issued patent by measuring the range of technology fields and, consequently, of industries that cite that patent [88, 89]. On the other hand, the originality index measures the range of technological fields in which a patent is based [90, 89].

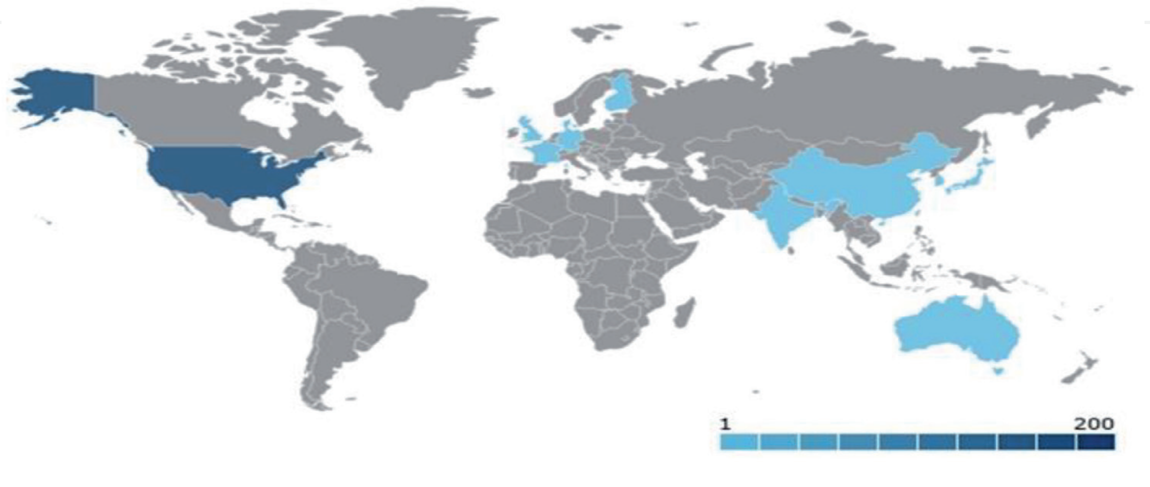

Figure 3.
Distribution of priority patent applications in the various offices over the last 20 years (1999–2019). Source: Research data from Questel-orbit platform.

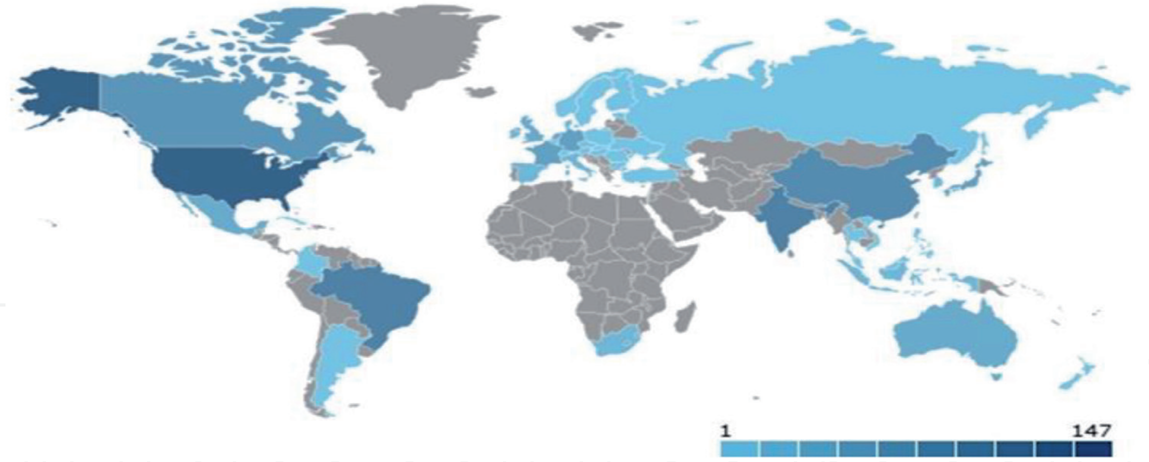

Figure 4.
Worldwide distribution of patents under protection by national patent offices over the last 20 years (1999–2019). Source: Research data from Questel-orbit platform.

Assignees	Active patent families	Average family size	Indicators	
			Originality	Generality
Novozymes	27	5,8	0,91	0,87
Du Pont De Nemours	18	8,7	0,87	0,87
Butamax Advanced Biofuels	17	9,3	0,90	0,88
Lallemand	14	5,7	0,85	0,84
DSM	11	6,5	0,84	0,81
Danisco	10	8,6	0,90	0,88
University of Florida	6	1,7	0,84	0,87
Toray Industries	9	7,1	0,92	0,84
DSM Ip Assets	7	7,4	0,83	0,78
University of California	3	2,7	0,86	0,88

Source: research data from Questel-Orbit platform.

Table 2.
Patent families by assignees and value indicators.

Novozymes, the largest patent holder (27 patent families), is a Danish company that develops and markets enzymes for industrial use. We also highlight the American companies DuPont De Nemours (18 families of patents) and Butamax Advanced Biofuels (17 families). Butamax emerged from the partnership of DuPont and BP, so that in 2017, it acquired the company Nesika Energy LLC, installing an ethanol production plant in Scandia County in the state of Kansas-US, to add to this unit the production of bio-isobutanol. The top five global companies holding patents on the analyzed technology include Canadian Lallemand with 14 patent families and the Dutch company DSM, which has 12 patent families. Regarding the average family size of patents, Butamax Advanced Biofuels is configured with the largest average family size, about 9.3, followed by DuPont De Nemours (8.7) and Danisco (8.6).

Butamax Advanced Biofuels (0.88), Danisco (0.88), the University of California (0.88), Novozymes (0.87), and Du Pont De Nemours (0.87) have the highest patent generality indices and, consequently, tend to account for the most relevant applications. Toray Industries (0.92) and Novozymes (0.91) show the highest scores for the originality index. The importance of the companies cited for inventions and subsequent innovations in the technological field analyzed is undeniable.

We emphasize that, except for Butamax Advanced Biofuels that aims at the production and commercialization of bio-isobutanol, the other companies aim at developing and commercializing enzymes, yeasts, and catalysts for the production of advanced biofuels.

3. Perspectives for future research and innovations

The present study gathers evidence of technological opportunities for ethanol production from raw materials derived from the bioeconomy. As evidenced in our findings, the development of innovations in this field requires multidisciplinary knowledge, providing solutions for industrial applications, which employ *S. cerevisiae*, *E. coli*, and *Z. mobilis* [91]. However, these potentially usable microorganisms in these fermentative processes are not naturally adaptable to extreme industrial conditions [92] or do not tolerate high concentrations of inhibitory compounds released during biomass fermentation [93]. Thus, to overcome these barriers, different synthetic biology and metabolic engineering approaches are employed to microorganisms to make them robust living factories adapted to the industrial activities required for biomass fermentation into ethanol [5, 94, 95]. These insights about synthetic biology may allow folding and probing the genome at different length and time scales, making it possible to understand gene positioning and functions [96]. Nevertheless, we check the prospect of new unconventional yeasts and bacteria such as *P. stipitis* for fermentation of lignocellulosic biomass.

Because the yeast *S. cerevisiae*, commonly used in ethanol fermentation of sugar-based feedstocks, is not a natural degrader of arabinose [97] and xylose [98, 99], making the fermentation processes accessible to these sugars requires pathway engineering [100, 101]. Ye et al. [102] integrated a heterologous fungal arabinose pathway into *S. cerevisiae*, with the deletion of the PHO13 phosphatase gene, increasing the rate of arabinose consumption and ethanol production under aerobic conditions. In Cunha et al. [103], two pathways (XR/XDH or XI) of xylose assimilation by *S. cerevisiae* were compared in ethanol production under different fermentation conditions, demonstrating satisfactory results for the feasibility of this fuel from non-detoxified hemicellulosic hydrolysates. Meanwhile, Mitsui et al. [104] developed a novel genome shuffling method using CRISPR-Cas to improve stress tolerance in S. cerevisiae yeast. Regarding *E. coli*, its main disadvantages refer to the narrow growth range of neutral pH (6.0–8.0), in addition to ethanol not being a core product for this bacterium. However, Sun et al. [105] successfully developed an efficient bioprocess using an *E. coli* strain for ethanol production and xylose recovery from corn cob hydrolysate. Strains of this bacterium with regulated glucose utilization showed efficient metabolism of mixed sugars in lignocellulosic hydrolysates, and higher ethanol production yields [106]. In the same perspective, metabolic engineering has been studied to provide simultaneous utilization of glucose and xylose in this bacterial culture [107].

High cellulosic ethanol yields are achieved using *Z. mobilis* strains due to their unique physiology [108, 109]. It is possible to employ other substrates, mitigating the socio-environmental challenges for expanding ethanol production [30, 110]. Different approaches have been tested in *Z. mobilis* to improve the fermentation of lignocellulosic biomass substrates into ethanol [111, 112].

One critical step in developing methods of the microbial fermentation process of lignocellulosic biomass is its pre-treatment to increase the digestibility of the available sugars. Lignocellulosic biomass consists of highly crystalline cellulose and a hemicellulose sheath wrapped in a lignin network. This structure causes recalcitrance in fermentation processes [113, 114]. Recalcitrance is the main obstacle to using lignocellulosic biomass for ethanol production. It determines the rest of the fermentation and the overall efficiency of the process [115]. Biological pre-treatment has been employed for the deconstruction of this biomass because of its wide application, lower energy consumption, no generation of toxic substances, and higher yield [116].

Co-fermentation of different sugars from lignocellulosic biomass and its residues enables ethanol production processes to become economically viable [117]. The potential of using a blend of *E. coli* strains and yeasts to rapidly ferment all sugars in pretreated biomass at high ethanol rates is presented by Wang et al. [118]. In the same perspective, Amoah et al. [119] developed a yeast with xylose assimilation capable of co-fermenting xylose and glucose in ionic liquid for ethanol production from lignocellulosic biomass. Advances are also in attempting to overcome obstacles and perturbations present in the degradation of lignin [120] and cellulose [121] using microbial consortium and genetic engineering via RNA-guided Cas9 in *S. cerevisiae* [21], *Candida glycerinogenes* [122], *Rhodosporidium toruloides* [123]. The results denote significant increases in stress tolerance of microorganisms in severe fermentative processes.

4. Market and policy opportunities for innovation

The continued development of synthetic biology R&D for cellulosic ethanol production depends on both the technical and economic feasibility of the solutions presented. In the analyzed period, approximately US$ 820 million was invested in synthetic biology research aimed at the development of advanced biofuels and bioproducts from microbial systems [124].

Despite being the second-largest ethanol producer in the world, Brazil does not own priority patents registered in synthetic biology for cellulosic ethanol production, becoming only a target market for other countries holding these technologies, like the USA. In Brazil, the unit cost per protected patent is very high, corresponding to approximately US$ 13,000 per patent. Besides the poor institutional environment for innovation, the unit cost of protection may be one of the reasons for the lack of patent applications by Brazilian assignees. According to Cicogna et al. [125], Brazil is an example of an infant industry that is slowly reaching maturity. In the same perspective, Kang et al. [126] point to the need for government policies that facilitate the development of promising renewable technologies, in addition to offering incentives for their commercialization.

In an attempt to change this situation, in 2017 a new national policy for biofuels was enacted by the Brazilian government, the RenovaBio, aiming to promote ethanol and biodiesel production from various sources available in the country [127]. Brazil, in the future, could become the largest producer of bio-based products when economic, logistical, regulatory, and political barriers are overcome [128]. Its territorial extension and diverse regional edaphoclimatic characteristics enable the country an intensive production of biomass for industrial biotechnology at a relatively lower cost compared to other locations that prospect synthetic microbial cells. Moreover, it is one of the world's leading food producers with agroindustry generating a significant amount of waste with potential for transformation into bioenergy, providing a new pathway for biofuel production not competing with food but biomass and agricultural waste [129].

The knowledge applied to the creation of new technologies in synthetic biology related to cellulosic ethanol comes mainly from companies that work in the development of enzymes and microorganisms for the transformation of biomass into ethanol and also in the production and commercialization of this biofuel. Companies seek, through patents, the commercial exploitation of these new technologies as they maximize their competitive advantages [130]. Industries operate in complex technological environments. Their technical knowledge is highly relevant to gain a competitive advantage. Therefore, companies cannot rely solely on their internal R&D units but also need to seek support from external sources of technology. To protect their inventions from third-party misuse, innovative companies seek patent protection [131, 132].

5. Concluding remarks

In this chapter, we examined the developments and applications of synthetic biology tools related to cellulosic ethanol by analyzing patents to investigate the current stage and dynamics of this technological field and its role as a proxy for a sustainable bioeconomy using non-food feedstocks. The findings are not necessarily only involved in the field of synthetic biology, but also in its numerous approaches that could circumscribe the development of cellulosic ethanol production worldwide. Our analyses provide a compilation of relevant patents, allowing us to understand, track, and project the role of synthetic biology in fostering solutions for the emerging sustainable bioeconomy, and enabling socio-market scenarios with this orientation.

Thinking about sustainable bioeconomy for energy generation, the use of synthetic biology tools may provide new living factories increasingly adapted to industrial processing technology, despite the decrease in the search for patent applications. Using the results from this study, synthetic or bioenergy engineers will be able to choose robust microorganisms capable of performing optimized fermentation processes or biomass processing methods, alleviating a bottleneck that limits the yields of bioenergy research. As these efforts mature, they can be expanded into biofuel production based on bioeconomic-nonfood substrates.

Overall, the research has provided approach for evaluating synthetic biology R&D performance related to cellulosic ethanol and bioeconomy. The results can help researchers quickly integrate into the field as they will easily understand the technological frontiers. The study also provides references for future energy research and policies that could proxy for a world focused on a more sustainable bioeconomy using non-food feedstocks. In addition, the text illustrated the importance of knowledge spillovers in R&D and signaled possibilities for future work. Deepening the understanding of cellular systems can raise the yield of low-cost carbon sources for cellulosic ethanol production. Integration of different generations of technologies may be an alternative to improve the total yields and make cellulosic ethanol economically viable.

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Conflict of interest

The authors inform they have no conflict of interest to declare.

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